Journal of Cancer Research and Practice xxx (2016) 1–4

Contents lists available at ScienceDirect

Journal of Cancer Research and Practice

journal homepage: http://www.journals.elsevier.com/journal-of-cancer-research-and-practice

Case Report

Durable response to programmed death-1 (PD-1) blockade in a metastatic gastric cancer patient with mismatch repair deficiency and microsatellite instability

Ting-Hui Wu [a], Emily Han-Chung Hsiue [a], Chang-Tsu Yuan [b], Li-Hui Tseng [c], Chia-Chi Lin [a], Kun-Huei Yeh [a, d, *]

[a] Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan
[b] Department of Pathology, National Taiwan University Hospital, Taipei, Taiwan
[c] Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan
[d] Department of Medicine, National Taiwan University Cancer Center Hospital, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form
19 October 2016
Accepted 7 November 2016
Available online xxx

Keywords:
Gastric cancer
Anti-PD-1
Mismatch repair deficiency
Microsatellite instability

ABSTRACT

Mismatch repair deficiency (dMMR) or microsatellite instability (MSI) has been reported as a predictive biomarker for responses to programmed death-1 (PD-1) blockade in metastatic colorectal cancer. A high response rate to anti-PD-1 therapy was observed in other cancer types with MSI. We report a chemotherapy-refractory metastatic gastric cancer patient with dMMR and MSI who responded remarkably well to pembrolizumab, a PD-1 monoclonal antibody. The satisfactory objective response has lasted for more than 24 months as of this report.

1. Introduction

Gastric cancer is a major cause of cancer deaths worldwide, particularly in East Asia.[1] Metastatic gastric cancer is associated with poor outcomes. The median overall survival of metastatic gastric cancer ranges from 9 to 13 months despite treated with systemic chemotherapy, anti-human epidermal growth factor receptor (HER) 2 therapy (trastuzumab) for HER2-positive disease, and anti-vascular endothelial growth factor receptor (VEGFR) 2 monoclonal antibody (ramucirumab) in second-line treatments.[2–4] Since 2011, a novel class of anticancer therapy, immune checkpoint blockade, has been introduced, leading to a paradigm shift in oncology. Patients with metastatic gastric cancer are among those with fatal diseases who may benefit from these agents.

Cancer cells can evade the human immune system by regulating immune checkpoint molecules. The programmed death 1 (PD-1) protein, an inhibitory immune checkpoint molecule that has been extensively studied, downregulates immune responses by binding to its ligands, PD-L1 and -L2.[5] Monoclonal antibodies against PD-1, including pembrolizumab and nivolumab, have been approved by the US Food and Drug Administration for treating melanoma, lung cancer, and lymphoma. PD-1 blockade is effective in a fraction of patients with metastatic gastric cancer.[6] Until now, an ideal biomarker for predicting the response to PD-1 blockade remains elusive in metastatic gastric cancer.

Mismatch repair-deficient cancers are rich in lymphocyte infiltration, which indicates an immune response.[7] This finding led to the hypothesis that such tumors more favorably respond to immune checkpoint blockade. Le et al reported an association between mismatch repair deficiency (dMMR) and the response to PD-1 blockade in metastatic colorectal cancer (CRC). Patients with mismatch repair-deficient CRC showed a substantially higher response rate to PD-1 blockade than did those with proficient mismatch repair (pMMR) function.[8] Several other dMRR cancers have shown high response rates to anti-PD-1 therapy.[9] Herein, we report a chemotherapy-refractory metastatic gastric cancer patient with dMMR and microsatellite instability (MSI) who showed a satisfactory response to pembrolizumab (formerly MK-3475), a

* Corresponding author. Department of Oncology, National Taiwan University Hospital, No. 7, Chung-Shan S. Rd., Taipei City 10002, Taiwan.
E-mail address: khyeh@ntu.edu.tw (K.-H. Yeh).
Peer review under responsibility of The Chinese Oncology Society.

http://dx.doi.org/10.1016/j.jcrpr.2016.11.001

humanized monoclonal IgG4 antibody against PD-1, for more than 24 months.

2. Case report

A 66-year-old man visited the outpatient clinic at National Taiwan University Hospital in September 2009 after experiencing difficulty in swallowing, particularly when consuming solid food, for 3 weeks. The patient did not report marked weight loss, vomiting, or pain in the chest and abdomen. He did not smoke cigarettes or consume alcohol. The patient mentioned no history of cancer in first and second-degree relatives. No remarkable findings were observed on initial examinations at the clinic. Esophagogastroduodenoscopy showed an ulcerative mass with irregular borders at the gastric cardia, with lower esophageal involvement. The epicenter of the tumor was greater than 5 cm away from the esophagogastric junction. Biopsy of the mass showed adenocarcinoma, diffusely positive for cytokeratine but negative for CK7, CK20, and CDX2. Serial imaging studies for staging did not reveal distant metastasis. The patient underwent thoracoscopic esophagectomy and gastric tube reconstruction (Ivor Lewis procedure) in October 2009. The pathological stage was pT4aN1M0, stage IIIA. Adjuvant chemotherapy with cisplatin (60 mg/m^2 on day 1 every 2 weeks) plus infusional 5-fluorouracil (5-FU) and leucovorin (2200 mg/m^2 and 300 mg/m,^{2} respectively, on day 1 every 2 weeks) was administered from December 2009 to March 2010. In February 2011, computed tomography (CT) of the abdomen revealed multiple nodular lesions at the peritoneum and mesentery, which were later histologically proven as metastatic adenocarcinoma. The patient received salvage chemotherapy with oxaliplatin (85 mg/m^2 on day 1) and capecitabine (1250 mg/m^2 BID on days 1–14 every 2 weeks) from June 2011 to March 2012. Treatment assessment in April 2011 revealed progressive disease, and second-line chemotherapy with paclitaxel (80 mg/m^2 on day 1) and infusional 5-FU plus leucovorin (2600 and 300 mg/m,^{2} respectively, on day 1) every 2 weeks was administered from May 2012. A new metastatic tumor in the spleen was observed in July 2012, and the patient underwent debulking surgery of the peritoneal tumors and splenectomy in August 2012, as the tumor was limited in the abdomen. Metastasis at S6 of the liver was reported in August 2013; therefore, the patient underwent radiofrequency ablation in August and November 2013. In February 2014, CT revealed a new lymphadenopathy at the right perivertebral space next to the aortic hiatus. The patient started receiving 10 mg/kg pembrolizumab every 2 weeks in April 2014.[6] Eight weeks after therapy initiation, the lymphadenopathy, which was considered the target lesion, shrunk from 4.8 cm (Fig. 1) to 3.2 cm, representing a 33% reduction of relative to the pretreatment size. In April 2016, the highest reduction (59%) of the target lesion was achieved (Fig. 2). The response to pembrolizumab was durable, lasting for more than 24 months, and is still ongoing. The treatment was well tolerated by the patient, and no adverse effects other than grade 1 fatigue, elevation of alkaline phosphatase, and hypothyroidism were observed during the treatment period. The patient completed 2 years of pembrolizumab treatment in April 2016. He is currently alive and is not receiving any active anticancer treatment.

Fig. 1. CT performed before pembrolizumab treatment. The largest diameter of the perivertebral tumor was 4.8 cm.

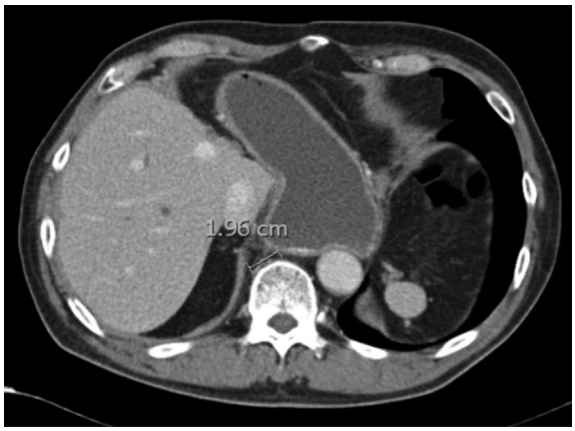

Fig. 2. CT performed at the time of the most favorable response after pembrolizumab treatment. The perivertebral tumor was hardly visible.

3. Analysis of mismatch repair function and results

Biopsy of the recurrent tumor at the perivertebral region was performed. MMR function was assessed by analyzing the microsatellite loci through polymerase chain reaction (PCR) and immunohistochemical (IHC) staining of proteins.

Analysis of microsatellite instability: Multiplex PCR amplification of 5 mononucleotide microsatellite loci was performed using the MSI Analysis System (Promega).[10] Amplification products were analyzed using the ABI 3500Dx capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA). The neoplasm is designated as having high MSI (MSI-H) when novel allele lengths are identified in the neoplastic cells compared with normal or germline cells at 2 or more microsatellite loci.[11]

Immunohistochemical staining of DNA mismatch repair proteins: IHC staining of MLH1 (clone ES05, 1:100; Dako), MSH2 (clone FE11, 1:100, Dako), PMS2 (clone EP51, 1:50, Dako), and MSH6 (clone EP49, 1:200, Dako) was performed using an automated immunostainer (BENCHMARK® XT, Ventana Medical System, Tucson, AZ, USA) and reviewed by 2 board-certified pathologists.

Results: MSI was observed in all 5 mononucleotide microsatellite loci compared with tumor tissues and peripheral blood, consistent with MSI-H (Fig. 3). IHC staining revealed that the tumor was negative for MLH1 and PMS2 (Fig. 4).

4. Discussion

Cancer cells can evade immune surveillance through several mechanisms. One of the most crucial mechanisms is suppressing immune responses by expressing immune checkpoints. Immune checkpoints have been extensively studied in the past few years, resulting in the development of immune checkpoint blockade.[12]

Pembrolizumab (formerly MK-3475), a humanized monoclonal

Please cite this article in press as: Wu T-H, et al., Durable response to programmed death-1 (PD-1) blockade in a metastatic gastric cancer patient with mismatch repair deficiency and microsatellite instability, Journal of Cancer Research and Practice (2016), http://dx.doi.org/10.1016/j.jcrpr.2016.11.001

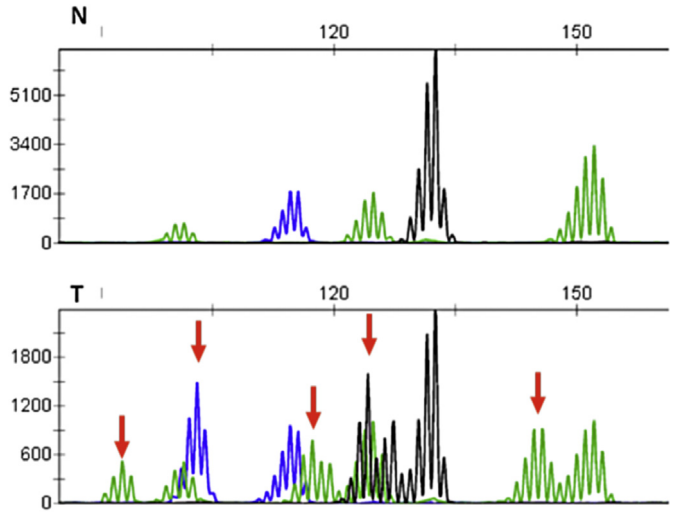

Fig. 3. MSI, MSI was observed in all 5 mononucleotide microsatellite loci, consistent with MSI-H. N: peripheral blood; T: tumor. Arrows indicate alleles with shifting.

and the median response duration was 24 weeks among the 36 enrolled patients who were treated with pembrolizumab.[6] PD-L1 appears to be a suboptimal predictive biomarker for PD-1 blockade in gastric cancer.

Virus-associated malignancies more favorably respond to immune checkpoint blockade. Higher expression of PD-L1 induced by viral infection and more tumor infiltrating lymphocytes in the microenvironment are hypothetic mechanisms underlying the more favorable responses.[20,21] Pembrolizumab is effective against virus-associated malignancies, such as nasopharyngeal carcinoma, Merkel cell carcinoma, and human papillomavirus-associated oropharyngeal cancer.[22] A small fraction of gastric adenocarcinoma is associated with Epstein–Barr virus (EBV).[23] However, the response to immune checkpoint blockade in EBV-associated gastric adenocarcinoma is unclear, and EBV-encoded small RNA is not established as a biomarker for immune checkpoint blockade in gastric cancer.

Deficient MMR (dMMR) causes MSI-H that results in a 10–100-fold increase in mutational load. Studies have reported that tumors with a high mutational load more favorably respond to immune

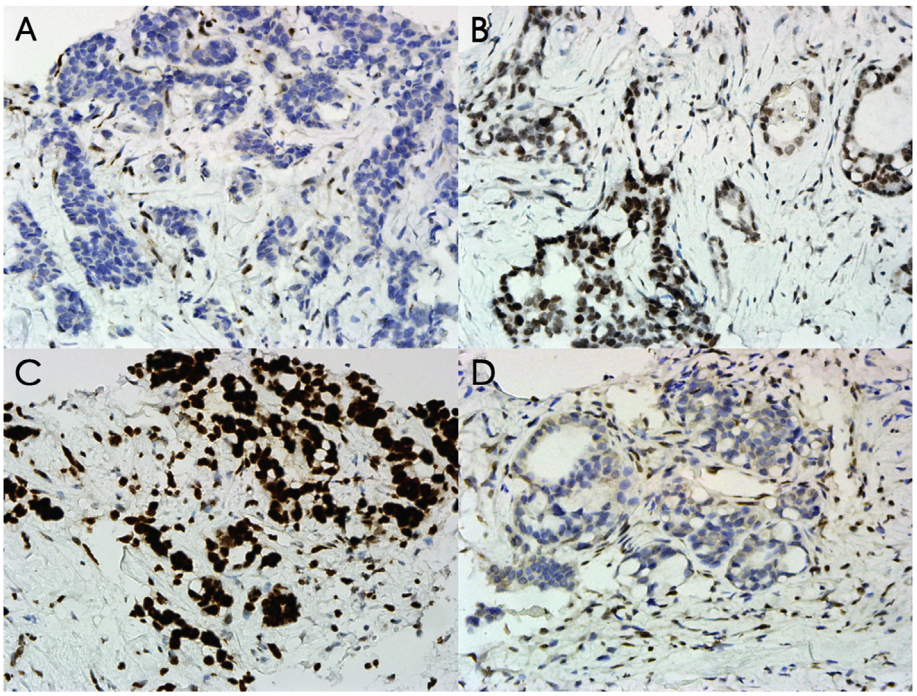

Fig. 4. IHC staining of (A) MLH1, (B) MSH2, (C) MSH6, and (D) PMS2 at 400 × Tumor cells lacked MLH1 and PMS2 MMR protein expression.

IgG4 antibody that targets immune checkpoint molecule: PD-1, has shown durable responses in melanoma, non-small- cell lung cancer, and several other cancer types.[13–15] Emerging data suggest that approximately 20% of patients also respond to PD-1 blockade.[9,16] However, identify a useful predictive biomarker for anti-PD-1 blockade in gastric cancer is challenging.

Programmed cell death ligand 1 (PD-L1) expression in tumor and invasive borders may potentially predict the response to PD-1 blockade; however, the predictability and optimal cutoff threshold remain controversial and inconclusive.[14,17–19] Muro et al reported the efficacy of pembrolizumab in pretreated patients with PD-L1 expressing (distinctive stromal or ≥1% tumor nest cell PD-L1 staining) recurrent or metastatic adenocarcinoma of the stomach or gastroesophageal junction. The objective response rate was 22%,

checkpoint blockade.[24,25] Moreover, Le et al revealed that metastatic colon cancers harboring MSI-H favorably respond to PD-1 blockade, with an objective response rate of 40%.[8]

Approximately 15%–20% of patients in all stages of gastric cancer demonstrate the MSI-H phenotype.[26,27] MSI-H is associated with favorable prognosis in gastric cancer,[28,29] whereas its predictability of the response to immune checkpoint blockade is controversial. At the annual meeting of the American Society of Clinical Oncology in 2016, Le et al reported the results of a subsequent cohort of various cancer types other than CRC with dMMR treated with pembrolizumab. Two of the three enrolled patients with advanced gastric cancer responded to PD-1 blockade; one had a complete response and one had a partial response.[9] In contrast, Chen et al reported a patient with MMR-proficient (pMMR) and

Please cite this article in press as: Wu T-H, et al., Durable response to programmed death-1 (PD-1) blockade in a metastatic gastric cancer patient with mismatch repair deficiency and microsatellite instability, Journal of Cancer Research and Practice (2016), http://dx.doi.org/10.1016/j.jcrpr.2016.11.001

microsatellite-stable (MSS) gastric cancer who exhibited a partial response to pembrolizumab. This result indicates that pMMR and an MSS status may not fully predict resistance to PD-1 blockade in gastric cancer, as suggested by a previous study on CRC by Le et al, in which none of the 18 MSS patients responded to PD-1 blockade.[8]

We herein report a patient with dMMR and MSI-H metastatic gastric cancer who responded favorably and durably to PD-1 blockade. This patient, along with the previous two demonstrated by Le et al,[9] are up-to-date the only 3 MMR gastric cancer patients who responded well to PD-1 blockade. Response to PD-1 blockade was also observed in patients with pMMR and MSS gastric cancer[30]; therefore, the clinical implications of MMR function in gastric cancer require further investigation.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136:E359–E386.
2. Catalano V, Labianca R, Beretta GD, et al. Gastric cancer. *Crit Rev Oncol Hematol*. 2009;71:127–164.
3. Bang Y-J, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomized controlled trial. *Lancet*. 2010;376:687–697.
4. Garon EB, Ciuleanu TE, Arrieta O, Prabhash K. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomized phase 3 trial. *Lancet*. 2014;384:665–673.
5. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252–264.
6. Muro K, Chung HC, Shankaran V, Geva R. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol*. 2016;17:717–726.
7. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. *Cancer*. 2001;91:2417–2422.
8. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509–2520.
9. Le DT, Uram JN, Wang H, et al. PD-1 blockade in mismatch repair deficient non-colorectal gastrointestinal cancers. *ASCO Meet Abstr*. 2016;34:195.
10. Tseng L-H, Tang J-L, Haley L, et al. Microsatellite instability confounds engraftment Analysis of hematopoietic stem-cell transplantation. *Appl Immunohistochem Mol Morphol*. 2014;22:416–420.
11. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*. 1998;58:5248–5257.
12. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;348:56–61.
13. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomized, controlled, phase 2 trial. *Lancet Oncol*. 2015;16:908–918.
14. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non–small-cell lung Cancer. *N Engl J Med*. 2015;372:2018–2028.
15. Patnaik A, Kang SP, Rasco D, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clin Cancer Res*. 2015;21:4286–4293.
16. Muro K, Chung HC, Shankaran V, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol*. 2016;17:717–726.
17. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res*. 2014;20:5064–5074.
18. Garon EG, Rizvi NA, Leighl NB, et al. 127 Correlation of clinical activity of pembrolizumab (MK-3475) with immunohistochemical staining for programmed death-1 ligand (PD-L1) in ≥50% of tumor cells in a prospective non-small cell lung cancer (NSCLC) validation population. *Eur J Cancer*. 2014;50:44–45.
19. Velcheti V, Schalper KA, Carvajal DE, et al. Programmed death ligand-1 expression in non-small cell lung cancer. *Lab Invest*. 2014;94:107–116.
20. Green MR, Rodig S, Juszczynski P, et al. Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy. *Clin Cancer Res*. 2012;18:1611–1618.
21. Sihto H, Joensuu H. Tumor-infiltrating lymphocytes and outcome in Merkel cell carcinoma, a virus-associated cancer. *OncoImmunology*. 2012;1:1420–1421.
22. Nghiem PT, Bhatia S, Lipson EJ, et al. PD-1 blockade with pembrolizumab in advanced Merkel-cell carcinoma. *N Engl J Med*. 2016;374:2542–2552.
23. Shibata D, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. *Am J Pathol*. 1992;140:769–774.
24. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. *Science*. 2015;348:124–128.
25. Chan TA, Wolchok JD, Snyder A. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2015;373, 1984–1984.
26. Ottini L, Falchetti M, Lupi R, et al. Patterns of genomic instability in gastric cancer: clinical implications and perspectives. *Ann Oncol*. 2006;17:vii97–vii102.
27. Network TCGAR. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513:202–209.
28. Giampieri R, Maccaroni E, Mandolesi A, et al. Mismatch repair deficiency may affect clinical outcome through immune response activation in metastatic gastric cancer patients receiving first-line chemotherapy. *Gastric Cancer*. 2016:1–8.
29. Marrelli D, Polom K, Pascale V, et al. Strong prognostic value of microsatellite instability in intestinal type non-cardia gastric Cancer. *Ann Surg Oncol*. 2015;23:943–950.
30. Chen K-H, Yuan C-T, Tseng L-H, Shun C-T, Yeh K-H. Case report: mismatch repair proficiency and microsatellite stability in gastric cancer may not predict programmed death-1 blockade resistance. *J Hematol Oncol*. 2016;9:29.

Please cite this article in press as: Wu T-H, et al., Durable response to programmed death-1 (PD-1) blockade in a metastatic gastric cancer patient with mismatch repair deficiency and microsatellite instability, Journal of Cancer Research and Practice (2016), http://dx.doi.org/10.1016/j.jcrpr.2016.11.001